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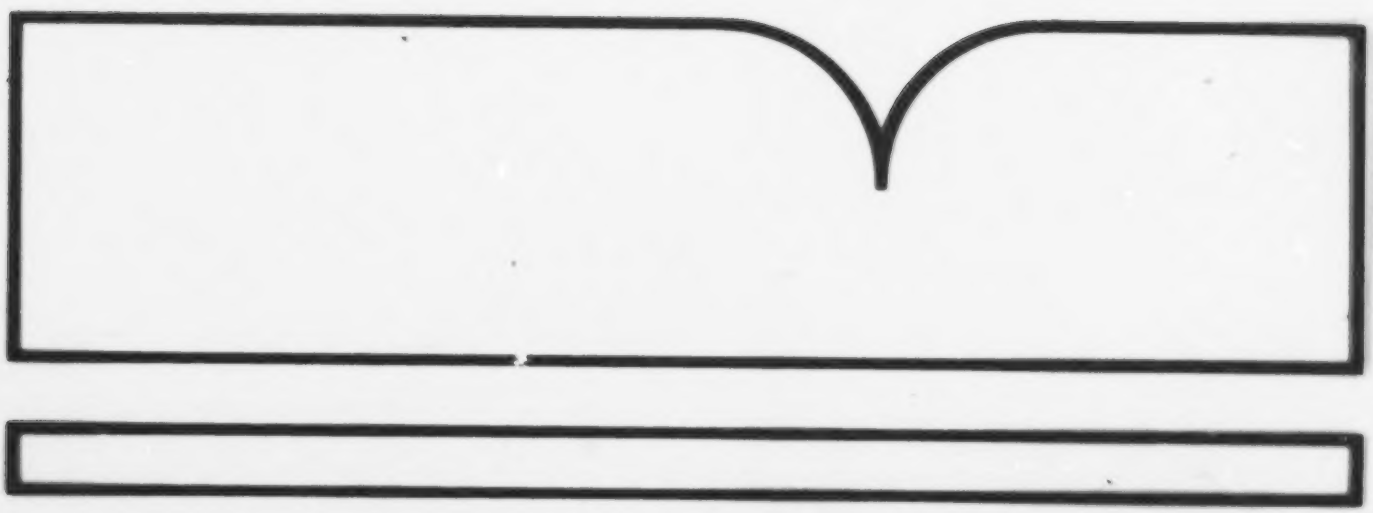

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PESTICIDE ASSESSMENT GUIDELINES

SUBDIVISION F

HAZARD EVALUATION:

HUMAN AND DOMESTIC ANIMALS

Series 81-3 and 82-4

Acute and Subchronic Inhalation Toxicity Testing

ADDENDUM 6 ON DATA REPORTING

Prepared by:

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for

Science Analysis and Coordination Branch
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Office of Pesticide Programs
US Environmental Protection Agency

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16. Abstract (Limit: 200 words)

The Data Reporting Guideline (DRG) for Acute and Subchronic Inhalation Toxicity Testing, Series 81-3 and 82-4, gives guidance to pesticide registrants on the format of their study report so that the Agency can review it efficiently. This guidance clarifies sections in the existing Pesticide Assessment Guidelines, Subdivision F, on data reporting. It provides an outline for the study report which data submitters can use in preparing their reports for submission to EPA to meet 40 CFR 158 data requirements for the registration of pesticides.

The DRG for Series 81-3 and 82-4, Acute and Subchronic Inhalation Toxicity Testing, is only one of a number of DRGs published by the National Technical Information Service as an addendum to Subdivision F of the Pesticide Assessment Guidelines.

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Subdivision F - Acute and Inhalation Toxicity Studies

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Acute Inhalation Toxicity

Guideline

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PESTICIDE ASSESSMENT GUIDELINES

HUMANS AND DOMESTIC ANIMALS

Acute and Subchronic Inhalation Toxicity Studies

Subdivision F, Series 81-3 and 82-4

DATA REPORTING

INTRODUCTION

A. Purpose

The purpose of the acute inhalation study is to determine the health hazards to animals from short-term exposure by the inhalation route. Subpart F, Series 81-3 provides guidelines for carrying out the acute inhalation toxicity study. The purpose of subchronic inhalation studies is to determine the health hazard to animals from exposure of intermediate duration by the inhalation route. Subpart F, Series 82-4 provides guidelines for carrying out subchronic inhalation toxicity studies.

B. Objective

The objective of these Data Reporting Guidelines (DRGs) is to provide an example of an acceptable general report format that can be reviewed effectively and expeditiously in accordance with the Agency's review process. The recommendations are intended to be consistent with the guidelines for data reporting as described in Sections 80-4, 81-3, and 82-4(h) and those described in the Agency's Good Laboratory Practice Standards (40 CFR Part 160).

While following this Guideline is not mandatory, data submitters are encouraged to submit complete reports which can be efficiently reviewed by the Agency. PR Notice 86-5, effective on November 1, 1986, and available from the Office of Pesticide Programs, EPA, pertains to the physical formatting of reports (which are referred to as "studies") and submittal packages. Some of its requirements are mandatory.

RESPONSE TO PUBLIC COMMENTS

The Agency received three responses to its request for public comments on these documents published in the FEDERAL REGISTER of March 25, 1987 (52 FR 9536). Some revisions were made in response to the comments. The following is the Agency's response to specific comments.

1. Comment:

Protocol deviations should be included in the text rather than in the summary.

Response:

The Data Reporting Guideline SUMMARY section requests only that any deviations from the intended protocol be noted. This request is for important major deviations that impact significantly on the results of the study and does not imply including a detailed listing of deviations which should, as suggested, be placed in the text (MATERIALS/METHODS) and discussed as appropriate in the DISCUSSION.

2. Comment:

If the Guideline reference is to be included on the cover page (per PR Notice 86-5), then the reference should not need to be repeated in the introduction.

Response:

The INTRODUCTIONs of the DRGs for inhalation testing do not specifically ask for this information. However, it might be noted here that the Agency has agreed to accept studies using guidelines from other sources, such as the OECD, in which case, the Subpart F designation should be on the cover page, designating which EPA Guideline study is being satisfied and the reference to the alternative protocol made in the INTRODUCTION.

3. Comment:

A detailed explanation of the experimental design should not be required in the Introduction.

Response:

The INTRODUCTION does not ask for such a detailed discussion of the experimental design. It recommends a brief comment on the overall experimental design as an introduction to the detail which is to follow.

4. Comment:

The composition and purity of water data should not be included in an inhalation study and is part of Good Laboratory Practices (GLP).

Response:

The water source, how it is offered to the animals inside of the chamber, etc., is and can be an important additional source of exposure to the test agent. Although water is generally offered

through a nipple watering system, this is not always the case. Water purity (especially relative to chemical inducing agents) is determined in most laboratories and should be mentioned as one of the GLP procedures for assuring good animal husbandry.

5. Comment:

Objections were made against providing data on the physical and chemical properties of the test agent. It was noted that the testing laboratory does not normally have the capabilities of determining these characteristics.

Response:

The toxicology testing laboratory is not expected to determine such properties as chemical stability, vapor pressure, and the like; however, the laboratory needs to and should have such information which may be supplied as a Material Safety Data Sheet as part of the information needed to develop an appropriate inhalation testing procedure. It aids in the review of the inhalation study if the Agency reviewer has such information readily available.

6. Comment:

The conduct of a limit test should not be considered a deviation as it is presented as a guideline option.

Response:

If there are no deaths at or above 5 mg/L of the test substance using 5 animals/sex, then further acute inhalation testing is not required. This limit is used to relieve investigators from meeting the requirements for a complete LC_{50} study when they are working with a relatively nontoxic agent and cannot get mortality at or below the limit concentration.

If an investigator feels that the test agent he is testing would produce no mortality at the 5 mg/L concentration, he/she can test the animals at the limit directly. This approach is therefore an "option," but should not be considered as a "deviation" as indicated by the commenter.

7. Comment:

The commenters indicated that it is not appropriate to analyze the test material in the diet in an inhalation study.

Response:

This is correct. A parenthetical phrase "(if the test material is administered in the diet)" was inadvertently included in the Guideline and has been deleted. Relative to the diet, however, the investigators

should identify the "diet (vendor and composition)" as noted in Section III. A. Test Animals referring to the routinely provided nutritional composition of the diet all of the animals receive. The laboratory or supplier provides this information and often provides the listing of contaminants which sometimes affect such studies.

8. Comment:

The commenters indicated that duplicating the protocol in the Appendix is unnecessary.

Response:

The Guideline lists a number of items which should be placed in an APPENDIX but did not suggest repeating the protocol as such. It did indicate that details of the protocol be put in the APPENDIX as "appropriate" (at the option of the investigator, implied).

9. Comment:

The commenter refers to §80-4(b)(7) of Subpart F Guidelines and indicates that this section would require a separate and unnecessary duplication of reporting in the report.

Response:

Section 80-4 of Subpart F is a section on Reporting of Data from §80, the Overview, Definition, and General Requirements. There is no article section (b)(7) in §80-4, however, Section 80-4(a)(2) discusses verification requirements. Data Reporting Guidelines for inhalation testing make reference to other portions of the Guidelines which discuss the same or related requirements in an effort to avoid duplication.

PESTICIDE ASSESSMENT GUIDELINES

HUMAN AND DOMESTIC ANIMALS

Acute Inhalation Toxicity Testing

Subdivision F, Series 81-3

DATA REPORTING

UIDELINE

The Final Report should contain the following items.

COVER PAGE

The title page and additional documentation (i.e., requirements for data submission, Good Laboratory Practices statement, and statement of data confidentiality claims), if relevant to the study report, must precede the content of the study format below. These requirements are described in PR Notice 86-5.

TABLE OF CONTENTS

The table of contents should include a listing of the elements of the final report such as the Summary, an Introduction, the Materials and Methods, Results, Discussion, Bibliography, Tables, Figures, Appendices, and key subsections as appropriate.

BODY OF THE REPORT

This item shall include all information required in §§80-4(b)(2), 80-4(c), and 81-3(h) of this subdivision and should be provided in such detail that the reviewer can assess the quality of the study and its conformity to the acute inhalation testing Guideline. It should contain the following sections.

I. SUMMARY

As per §80-4(b)(1) of this Subdivision, the test report shall contain a summary including a brief description of the study protocol, chemical used, the animals tested, and highlights of the results of the study relative to the positive findings. Any deviations from the intended protocols should be noted.

II. INTRODUCTION

Include the objectives of the study and the Guideline reference. The overall experimental design should be explained including the type of exposure system (usually whole body exposures in dynamic chambers), the species used, the duration of exposure and post-exposure period, and the type of assessment (primarily the determination of the LC_{50}).

III. MATERIALS AND METHODS

This section of the report shall include information required by §80-4 of Subdivision F as well as the following information.

A. Test Animals

This section should identify:

1. Species and strain;
2. Source of animals;
3. Age and/or body weight;
4. Rationale for use of this species;
5. Preconditions (quarantine, chamber acclimation);
6. Methods of assignment to the experimental study; and
7. Animal care (apart from handling animals in exposure chambers) including;

    a. Housing,
    b. Diet (vendor and composition), and
    c. Water source and purity.

B. Test Substance

1. Besides that information requested in §80-4, this section should provide and identify:

    a. The chemical stability especially in air (relative to temperature and humidity);
    b. Solubility (in aqueous and organic solvents); and
    c. Physical properties, including melting point (solids), boiling point (liquids), vapor pressure, and flammability.

2. This section should also identify in detail the test agent describing:

    a. The type of test material (technical, manufacturing-use product, or formulation);
    b. Its composition;
    c. Physical properties;
    d. Source; and
    e. Batch or lot number.

3. If a vehicle or carrier is used during the generation of the test atmosphere, the composition and source of this material should be described in detail.

C. Experimental Design

This section should describe how the animals were assigned to the test groups, what exposure periods were used, the post-exposure periods, etc., consistent with the test Guidelines (§81-3).

D. Test Exposures

1. Exposure Chambers

This section should provide sufficient information on the exposure chambers to allow the reviewer to evaluate the suitability of the chambers used. This should include such information as the construction, the dimensions, and sources of the chambers used. If the chambers are not commercially available, then information should be provided on the design features and preliminary testing which ensures uniform distribution of air within the chambers. Any alarm systems and automatic recording devices need to be described. The source of make-up air including treatment (filtration, heating and cooling, and humidity control) should be described.

2. Chamber Monitoring

This should include information on the measurements of flow through the chambers, their temperature and relative humidity. Concentration and particle sizing are considered below. The equipment used to measure these parameters needs to be identified as to the vendor, model number, and the method of measurement and recording. Any procedures and equipment used to calibrate the equipment used should be described. The frequency of measurement will depend on the type of study—whether it is an acute study or a repeat exposure study.

3. Generating System

The generating systems used for introducing test materials into chambers will vary with the physical state of the test agent and can involve a wide variety of devices ranging from simple metering devices for gases, evaporating dishes or bubbling bottles for liquid vapors, and nebulizers for liquid aerosols, to particle generators for solid aerosols. The equipment used (vendor, model numbers, dimensions, construction details, etc.), should be described. Problems that arise during development and calibration of the generating system should be discussed in some detail, especially if difficulty is encountered in attaining the necessary concentrations within the chambers.

4. Chamber Concentrations

This should include:

a. The sampling sites within the chamber, the sampling devices used, and the frequency of analyses.
b. The analytical methods used to determine the concentrations of the test agent within the chamber should be discussed in detail (with references).
c. The sampling and analytical equipment used should be described as to vendor, model, source, etc.

d. Any chemicals used in the process should be described as to purity, source, preparation and the like.
e. Information should be provided on the overall precision, accuracy, specificity, and reliability of the chosen methods of analyses for chamber concentrations.

5. Particle Size Determinations

Particle size determinations need to be made when test atmospheres are generated as aerosols (sprays or dusts). A description of the methods and equipment used to characterize the aerosols should include such information as the sampling sites, the sampling system, sampling frequency and duration, and the instruments for particle sizing (usually cascade impactors), giving vendors, models, sources, etc. Details on the methods for calibrating and standardizing the sampling flow rates need to be included.

E. Evaluation Procedures

The Final Report should describe the types of observations of the animals for toxic signs, body weights, the frequency of observations, the gross pathological examination procedures and, if applicable, the methods used for histopathological examination consistent with the requirements in Subpart §81-3(9) to (11). The statistical methods used to calculate the LC_{50}, the 95 percent confidence limits, and the slope of the dose-response curves should be specified and referenced.

F. Deviations from Protocol

A description of the rationale for deviations from the protocol recommended in §81-3 should be provided along with the rationale for changes made. If the reported data are based on a limit test (§81-3(f)), the report should state why it was not possible to perform the complete test with possible explanations for the differences.

IV. RESULTS

This section should provide a summary of the toxic signs, mortality, body weight changes, gross pathology and, if obtained, histopathology based on the test levels used. Typically, these results are summarized in tabular form.

V. DISCUSSION

This section should provide an assessment of the results of the study, an interpretation of toxicity observed, the consistency of the dose-response effects and should try to explain unexpected findings. The impact of any deviations from the expected protocol should be discussed including an assessment of the adequacy of the test for the intended purposes.

VI. BIBLIOGRAPHY

This item should contain a list of references cited in the body of the report.

VII. VERIFICATION

This item shall contain information required by §80-4(b)(2). Each test report shall be:

A. Signed by each of the senior scientific personnel, including the laboratory director responsible for performing and supervising the testing, preparing, reviewing, and approving of the test report; and

B. Certified by the applicant or an authorized agent of the applicant as a complete and unaltered copy of the report provided by the testing laboratory whether independent or owned, operated, or controlled by the applicant.

VIII. ARCHIVES

This section of the Final Report shall contain all information required in 40 CFR 160.185.

IX. TABLES/FIGURES

X. APPENDIX(ES)

These should include animal data, historical control data, pathology report, analytical methods, and results of analyses on the test substance and test diet, details of statistical analyses, protocol, and other information as appropriate.

PESTICIDE ASSESSMENT GUIDELINES

HUMANS AND DOMESTIC ANIMALS

Subchronic Inhalation Toxicity Study

Subdivision F, Series 82-4

DATA REPORTING

The subchronic inhalation study (Subpart F, Series §82-3) discussed here represents a repeat inhalation exposure protocol which usually extends over a 90-day exposure period. The exposure period of a subchronic inhalation study may be shortened (usually to 30 days) or extended (usually to 6 months) depending on the purpose of the study. The inhalation exposure techniques reporting requirements discussed here for the subchronic inhalation studies apply to other toxicity studies described in Subpart F, series §83, where the inhalation route is used, namely, chronic and/or oncogenicity studies, reproduction or teratology studies. Differences between these studies will be in the numbers of animals used, the types of observations required, the inclusion of interim sacrifice groups, etc. The additional requirements are specified by the appropriate Guidelines; however, the inhalation testing aspects of each of these studies should be reported in the same format as presented here.

GUIDELINE

The Final Report should contain the following items.

COVER PAGES

The title page and additional documentation (i.e., requirements for data submission, Good Laboratory Practices statements, and statement of data confidentiality claims), if relevant to the study report, must precede the content of the study format below. These requirements are described in PR Notice 86-5.

TABLE OF CONTENTS

The table of contents should include a listing of the elements of the final report such as the Summary, an Introduction, the Materials and Methods, Results, Discussion, Bibliography, Tables, Figures, Appendices and key subsections as appropriate.

BODY OF THE REPORT

This item shall include all information required in §§80-4(b)(2), 80-4(c), and 82-4(h) of this subdivision and should be provided in such detail that the reviewer can assess the quality of the study and its conformity to the Subchronic Inhalation Testing Guideline. It should contain the following sections.

I. SUMMARY

As per §80-4(b)(1) of this Subdivision, the test report shall contain a summary including a brief description of the study protocol, chemical used, the animals tested, and highlights of the results of the study relative to the positive findings. Any deviations from the intended protocols should be noted.

II. INTRODUCTION

Include the objectives of the study and the Guideline reference. The overall experimental design should be explained including the type of exposure system (usually whole body exposures in dynamic chambers), the species used, the duration of exposure and post-exposure period, interim sacrifice times, and the type of assessment (primarily a study of indeterminate duration).

III. MATERIALS AND METHODS

This section of the report shall include information required by §80-4 of Subdivision F as well as the following information.

A. Test Animals

This section should identify:

1. Species and strain;
2. Source of animals;
3. Age and/or body weight;
4. Rationale for use of this species;
5. Preconditions (quarantine, chamber acclimation);
6. Methods of assignment to the experimental study; and
7. Animal care (apart from handling animals in exposure chambers) including;

    a. Housing,
    b. Diet (vendor and composition), and
    c. Water source and purity.

B. Test Substance

1. Besides that information requested in §80-4, this section should identify:

    a. The chemical name of the active ingredient, Chemical Abstract Number and code numbers;
    b. Chemical stability especially in air (relative to temperature and humidity);
    c. Solubility (in aqueous and organic solvents); and
    d. Physical properties including melting point (solids), boiling point (liquids), vapor pressure, and flammability.

2. This section should also identify in detail the test agent used to generate the test atmospheres:

   a. Its composition;
   b. Physical Properties;
   c. Source of procurement; and
   d. Batch or lot number of each procurement.

3. Any vehicle or carrier used to enable the test material to be generated should be described in detail.

C. Experimental Design

This section should describe the methods for assigning animals to the different test groups including the controls, the low, middle, and high test groups; the rationale for the levels chosen; the planned interim sacrifices; etc., consistent with the test Guidelines (§82-4).

D. Test Exposures

1. Exposure Chambers

This section should provide sufficient information on the exposure chambers to allow the reviewer to evaluate the suitability of the chambers used. This should include such information as the construction, the dimensions, and sources of the chambers used. If the chambers are not commercially available ones, then information should be provided on the design features and preliminary testing which ensures uniform distribution of air within the chambers. Any alarm systems and automatic recording devices need to be described. The source of make-up air including treatment (filtration, heating and cooling, and humidity control) should be described.

2. Chamber Monitoring

This should include information on the measurements of flow through the chambers, their temperature and relative humidity. Concentration and particle sizing are considered below. The equipment used to measure these parameters needs to be identified as to the vendor, model number, and the method of measurement and recording. Any procedures and equipment used to calibrate the equipment used should be described. The frequency of measurement will depend on the type of study--whether it is a subchronic study or a repeat exposure study.

3. Generating System

The generating systems used for introducing test materials into chambers will vary with the physical state of the test

agent and can involve a wide variety of devices ranging from simple metering devices for gases, evaporating dishes or bubbling bottles for liquid vapors, and nebulizers for liquid aerosols, to particle generators for solid aerosols. The equipment used (vendor, model numbers, dimensions, construction details, etc.), should be described. Problems that arise during development and calibration of the generating system should be discussed in some detail, especially if difficulty is encountered in attaining the necessary concentrations within the chambers.

4. Chamber Concentrations

This should include:

a. The sampling sites within the chamber, the sampling devices used, and the frequency of analyses.
b. The analytical methods used to determine the concentrations of the test agent within the chamber should be discussed in detail (with references).
c. The sampling and analytical equipment used should be described as to vendor, model, source, etc.
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5. Particle Size Determination

Particle size determinations need to be made when test atmospheres are generated as aerosols (sprays or dusts). A description of the methods and equipment used to characterize the aerosols should include such information as the sampling sites, the sampling system, sampling frequency and duration, and the instruments for particle sizing (usually cascade impactors), giving vendors, models, sources, etc. Details on the methods for calibrating and standardizing the sampling flow rates need to be included.

E. Evaluation Procedures

The Final Report should describe the types of observations of the animals. This should include the types of observations and analyses as described in the Guidelines (§82-4(9) through (14)) and should therefore include the frequency of observations, body weights, food consumption, mortality, the hematological examinations, clinical chemistries, urinalysis, gross pathological examination procedures, organ weight measurements and histopathological examination determined at each of the sacrifice times. The statistical analyses used to assess the data for each of the parameters evaluated should be discussed and referenced.

F. Deviations from Protocol

A description of the rationale for deviations from the protocol recommended in §82-4 should be provided along with the rationale for changes made.

IV. RESULTS

This section should provide a systematic summary of the observations made throughout the study summarizing the exposure conditions within each of the chambers and the effects each exposure regimen had on the animals. The data should be presented in graphic and tabular form whenever appropriate, indicating the statistical significance between exposure groups.

V. DISCUSSION

This section should provide an assessment of the results of the study, an interpretation of toxicity observed, the consistency of the dose-response effects and should try to explain unexpected findings. This should include the relationship between the concentration of the test substance and duration of exposure, and the presence or the absence, the incidence and severity, of abnormalities, including behavioral and clinical abnormalities, gross lesions, identified target organs, body weight changes, effects on mortality, and other general and specific toxic effects. This should include the basis for ascribing no-observable-effect and lowest-effect levels when observed. The impact of any deviations from the expected protocol should be discussed including as assessment of the adequacy of the test for the intended purposes.

VI. BIBLIOGRAPHY

This item should contain a list of references cited in the body of the report.

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This item shall contain information required by §80-4(b)(2). Each test report shall be:

A. Signed by each of the senior scientific personnel, including the laboratory director responsible for performing and supervising the testing, preparing, reviewing, and approving of the test report; and

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These should include individual animal data, historical control data, pathology report, analytical methods, and results of analyses on the test substance and test diet, details of statistical analyses, protocol, and other information as appropriate.

PESTICIDE ASSESSMENT GUIDELINES

SUBDIVISION F

HAZARD EVALUATION:

HUMAN AND DOMESTIC ANIMALS

Series 81-3 and 82-4

Acute and Subchronic Inhalation Toxicity Testing

ADDENDUM 6 ON DATA REPORTING

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for

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Elizabeth M. Leovey, PhD
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Subdivision F - Acute and Inhalation Toxicity Studies

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Acute Inhalation Toxicity

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PESTICIDE ASSESSMENT GUIDELINES

HUMANS AND DOMESTIC ANIMALS

Acute and Subchronic Inhalation Toxicity Studies

Subdivision F, Series 81-3 and 82-4

DATA REPORTING

INTRODUCTION

A. Purpose

The purpose of the acute inhalation study is to determine the health hazards to animals from short-term exposure by the inhalation route. Subpart F, Series 81-3 provides guidelines for carrying out the acute inhalation toxicity study. The purpose of subchronic inhalation studies is to determine the health hazard to animals from exposure of intermediate duration by the inhalation route. Subpart F, Series 82-4 provides guidelines for carrying out subchronic inhalation toxicity studies.

B. Objective

The objective of these Data Reporting Guidelines (DRGs) is to provide an example of an acceptable general report format that can be reviewed effectively and expeditiously in accordance with the Agency's review process. The recommendations are intended to be consistent with the guidelines for data reporting as described in Sections 80-4, 81-3, and 82-4(h) and those described in the Agency's Good Laboratory Practice Standards (40 CFR Part 160).

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2. Comment:

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Response:

The INTRODUCTIONs of the DRGs for inhalation testing do not specifically ask for this information. However, it might be noted here that the Agency has agreed to accept studies using guidelines from other sources, such as the OECD, in which case, the Subpart F designation should be on the cover page, designating which EPA Guideline study is being satisfied and the reference to the alternative protocol made in the INTRODUCTION.

3. Comment:

A detailed explanation of the experimental design should not be required in the Introduction.

Response:

The INTRODUCTION does not ask for such a detailed discussion of the experimental design. It recommends a brief comment on the overall experimental design as an introduction to the detail which is to follow.

4. Comment:

The composition and purity of water data should not be included in an inhalation study and is part of Good Laboratory Practices (GLP).

Response:

The water source, how it is offered to the animals inside of the chamber, etc., is and can be an important additional source of exposure to the test agent. Although water is generally offered

through a nipple watering system, this is not always the case. Water purity (especially relative to chemical inducing agents) is determined in most laboratories and should be mentioned as one of the GLP procedures for assuring good animal husbandry.

5. Comment:

Objections were made against providing data on the physical and chemical properties of the test agent. It was noted that the testing laboratory does not normally have the capabilities of determining these characteristics.

Response:

The toxicology testing laboratory is not expected to determine such properties as chemical stability, vapor pressure, and the like; however, the laboratory needs to and should have such information which may be supplied as a Material Safety Data Sheet as part of the information needed to develop an appropriate inhalation testing procedure. It aids in the review of the inhalation study if the Agency reviewer has such information readily available.

6. Comment:

The conduct of a limit test should not be considered a deviation as it is presented as a guideline option.

Response:

If there are no deaths at or above 5 mg/L of the test substance using 5 animals/sex, then further acute inhalation testing is not required. This limit is used to relieve investigators from meeting the requirements for a complete LC_{50} study when they are working with a relatively nontoxic agent and cannot get mortality at or below the limit concentration.

If an investigator feels that the test agent he is testing would produce no mortality at the 5 mg/L concentration, he/she can test the animals at the limit directly. This approach is therefore an "option," but should not be considered as a "deviation" as indicated by the commenter.

7. Comment:

The commenters indicated that it is not appropriate to analyze the test material in the diet in an inhalation study.

Response:

This is correct. A parenthetical phrase "(if the test material is administered in the diet)" was inadvertently included in the Guideline and has been deleted. Relative to the diet, however, the investigators

should identify the "diet (vendor and composition)" as noted in Section III. A. Test Animals referring to the routinely provided nutritional composition of the diet all of the animals receive. The laboratory or supplier provides this information and often provides the listing of contaminants which sometimes affect such studies.

8. Comment:

The commenters indicated that duplicating the protocol in the Appendix is unnecessary.

Response:

The Guideline lists a number of items which should be placed in an APPENDIX but did not suggest repeating the protocol as such. It did indicate that details of the protocol be put in the APPENDIX as "appropriate" (at the option of the investigator, implied).

9. Comment:

The commenter refers to §80-4(b)(7) of Subpart F Guidelines and indicates that this section would require a separate and unnecessary duplication of reporting in the report.

Response:

Section 80-4 of Subpart F is a section on Reporting of Data from §80, the Overview, Definition, and General Requirements. There is no article section (b)(7) in §80-4, however, Section 80-4(a)(2) discusses verification requirements. Data Reporting Guidelines for inhalation testing make reference to other portions of the Guidelines which discuss the same or related requirements in an effort to avoid duplication.

PESTICIDE ASSESSMENT GUIDELINES

HUMAN AND DOMESTIC ANIMALS

Acute Inhalation Toxicity Testing

Subdivision F, Series 81-3

DATA REPORTING

GUIDELINE

The Final Report should contain the following items.

COVER PAGE

The title page and additional documentation (i.e., requirements for data submission, Good Laboratory Practices statement, and statement of data confidentiality claims), if relevant to the study report, must precede the content of the study format below. These requirements are described in PR Notice 86-5.

TABLE OF CONTENTS

The table of contents should include a listing of the elements of the final report such as the Summary, an Introduction, the Materials and Methods, Results, Discussion, Bibliography, Tables, Figures, Appendices, and key subsections as appropriate.

BODY OF THE REPORT

This item shall include all information required in §§80-4(b)(2), 80-4(c), and 81-3(h) of this subdivision and should be provided in such detail that the reviewer can assess the quality of the study and its conformity to the acute inhalation testing Guideline. It should contain the following sections.

I. SUMMARY

As per §80-4(b)(1) of this Subdivision, the test report shall contain a summary including a brief description of the study protocol, chemical used, the animals tested, and highlights of the results of the study relative to the positive findings. Any deviations from the intended protocols should be noted.

II. INTRODUCTION

Include the objectives of the study and the Guideline reference. The overall experimental design should be explained including the type of exposure system (usually whole body exposures in dynamic chambers), the species used, the duration of exposure and post-exposure period, and the type of assessment (primarily the determination of the LC_{50}).

III. MATERIALS AND METHODS

This section of the report shall include information required by §80-4 of Subdivision F as well as the following information.

A. Test Animals

This section should identify:

1. Species and strain;
2. Source of animals;
3. Age and/or body weight;
4. Rationale for use of this species;
5. Preconditions (quarantine, chamber acclimation);
6. Methods of assignment to the experimental study; and
7. Animal care (apart from handling animals in exposure chambers) including;

    a. Housing,
    b. Diet (vendor and composition), and
    c. Water source and purity.

B. Test Substance

1. Besides that information requested in §80-4, this section should provide and identify:

    a. The chemical stability especially in air (relative to temperature and humidity);
    b. Solubility (in aqueous and organic solvents); and
    c. Physical properties, including melting point (solids), boiling point (liquids), vapor pressure, and flammability.

2. This section should also identify in detail the test agent describing:

    a. The type of test material (technical, manufacturing-use product, or formulation);
    b. Its composition;
    c. Physical properties;
    d. Source; and
    e. Batch or lot number.

3. If a vehicle or carrier is used during the generation of the test atmosphere, the composition and source of this material should be described in detail.

C. Experimental Design

This section should describe how the animals were assigned to the test groups, what exposure periods were used, the post-exposure periods, etc., consistent with the test Guidelines (§81-3).

D. Test Exposures

1. Exposure Chambers

This section should provide sufficient information on the exposure chambers to allow the reviewer to evaluate the suitability of the chambers used. This should include such information as the construction, the dimensions, and sources of the chambers used. If the chambers are not commercially available, then information should be provided on the design features and preliminary testing which ensures uniform distribution of air within the chambers. Any alarm systems and automatic recording devices need to be described. The source of make-up air including treatment (filtration, heating and cooling, and humidity control) should be described.

2. Chamber Monitoring

This should include information on the measurements of flow through the chambers, their temperature and relative humidity. Concentration and particle sizing are considered below. The equipment used to measure these parameters needs to be identified as to the vendor, model number, and the method of measurement and recording. Any procedures and equipment used to calibrate the equipment used should be described. The frequency of measurement will depend on the type of study--whether it is an acute study or a repeat exposure study.

3. Generating System

The generating systems used for introducing test materials into chambers will vary with the physical state of the test agent and can involve a wide variety of devices ranging from simple metering devices for gases, evaporating dishes or bubbling bottles for liquid vapors, and nebulizers for liquid aerosols, to particle generators for solid aerosols. The equipment used (vendor, model numbers, dimensions, construction details, etc.), should be described. Problems that arise during development and calibration of the generating system should be discussed in some detail, especially if difficulty is encountered in attaining the necessary concentrations within the chambers.

4. Chamber Concentrations

This should include:

a. The sampling sites within the chamber, the sampling devices used, and the frequency of analyses.
b. The analytical methods used to determine the concentrations of the test agent within the chamber should be discussed in detail (with references).
c. The sampling and analytical equipment used should be described as to vendor, model, source, etc.

d. Any chemicals used in the process should be described as to purity, source, preparation and the like.
e. Information should be provided on the overall precision, accuracy, specificity, and reliability of the chosen methods of analyses for chamber concentrations.

5. Particle Size Determinations

Particle size determinations need to be made when test atmospheres are generated as aerosols (sprays or dusts). A description of the methods and equipment used to characterize the aerosols should include such information as the sampling sites, the sampling system, sampling frequency and duration, and the instruments for particle sizing (usually cascade impactors), giving vendors, models, sources, etc. Details on the methods for calibrating and standardizing the sampling flow rates need to be included.

E. Evaluation Procedures

The Final Report should describe the types of observations of the animals for toxic signs, body weights, the frequency of observations, the gross pathological examination procedures and, if applicable, the methods used for histopathological examination consistent with the requirements in Subpart §81-3(9) to (11). The statistical methods used to calculate the LC_{50}, the 95 percent confidence limits, and the slope of the dose-response curves should be specified and referenced.

F. Deviations from Protocol

A description of the rationale for deviations from the protocol recommended in §81-3 should be provided along with the rationale for changes made. If the reported data are based on a limit test (§81-3(f)), the report should state why it was not possible to perform the complete test with possible explanations for the differences.

IV. RESULTS

This section should provide a summary of the toxic signs, mortality, body weight changes, gross pathology and, if obtained, histopathology based on the test levels used. Typically, these results are summarized in tabular form.

V. DISCUSSION

This section should provide an assessment of the results of the study, an interpretation of toxicity observed, the consistency of the dose-response effects and should try to explain unexpected findings. The impact of any deviations from the expected protocol should be discussed including an assessment of the adequacy of the test for the intended purposes.

VI. BIBLIOGRAPHY

This item should contain a list of references cited in the body of the report.

VII. VERIFICATION

This item shall contain information required by §80-4(b)(2). Each test report shall be:

A. Signed by each of the senior scientific personnel, including the laboratory director responsible for performing and supervising the testing, preparing, reviewing, and approving of the test report; and

B. Certified by the applicant or an authorized agent of the applicant as a complete and unaltered copy of the report provided by the testing laboratory whether independent or owned, operated, or controlled by the applicant.

VIII. ARCHIVES

This section of the Final Report shall contain all information required in 40 CFR 160.185.

IX. TABLES/FIGURES

X. APPENDIX(ES)

These should include animal data, historical control data, pathology report, analytical methods, and results of analyses on the test substance and test diet, details of statistical analyses, protocol, and other information as appropriate.

PESTICIDE ASSESSMENT GUIDELINES

HUMANS AND DOMESTIC ANIMALS

Subchronic Inhalation Toxicity Study

Subdivision F, Series 82-4

DATA REPORTING

The subchronic inhalation study (Subpart F, Series §82-3) discussed here represents a repeat inhalation exposure protocol which usually extends over a 90-day exposure period. The exposure period of a subchronic inhalation study may be shortened (usually to 30 days) or extended (usually to 6 months) depending on the purpose of the study. The inhalation exposure techniques reporting requirements discussed here for the subchronic inhalation studies apply to other toxicity studies described in Subpart F, series §83, where the inhalation route is used, namely, chronic and/or oncogenicity studies, reproduction or teratology studies. Differences between these studies will be in the numbers of animals used, the types of observations required, the inclusion of interim sacrifice groups, etc. The additional requirements are specified by the appropriate Guidelines; however, the inhalation testing aspects of each of these studies should be reported in the same format as presented here.

GUIDELINE

The Final Report should contain the following items.

COVER PAGES

The title page and additional documentation (i.e., requirements for data submission, Good Laboratory Practices statements, and statement of data confidentiality claims), if relevant to the study report, must precede the content of the study format below. These requirements are described in PR Notice 86-5.

TABLE OF CONTENTS

The table of contents should include a listing of the elements of the final report such as the Summary, an Introduction, the Materials and Methods, Results, Discussion, Bibliography, Tables, Figures, Appendices and key subsections as appropriate.

BODY OF THE REPORT

This item shall include all information required in §§80-4(b)(2), 80-4(c), and 82-4(h) of this subdivision and should be provided in such detail that the reviewer can assess the quality of the study and its conformity to the Subchronic Inhalation Testing Guideline. It should contain the following sections.

I. SUMMARY

As per §80-4(b)(1) of this Subdivision, the test report shall contain a summary including a brief description of the study protocol, chemical used, the animals tested, and highlights of the results of the study relative to the positive findings. Any deviations from the intended protocols should be noted.

II. INTRODUCTION

Include the objectives of the study and the Guideline reference. The overall experimental design should be explained including the type of exposure system (usually whole body exposures in dynamic chambers), the species used, the duration of exposure and post-exposure period, interim sacrifice times, and the type of assessment (primarily a study of indeterminate duration).

III. MATERIALS AND METHODS

This section of the report shall include information required by §80-4 of Subdivision F as well as the following information.

A. Test Animals

This section should identify:

1. Species and strain;
2. Source of animals;
3. Age and/or body weight;
4. Rationale for use of this species;
5. Preconditions (quarantine, chamber acclimation);
6. Methods of assignment to the experimental study; and
7. Animal care (apart from handling animals in exposure chambers) including;

    a. Housing,
    b. Diet (vendor and composition), and
    c. Water source and purity.

B. Test Substance

1. Besides that information requested in §80-4, this section should identify:

    a. The chemical name of the active ingredient, Chemical Abstract Number and code numbers;
    b. Chemical stability especially in air (relative to temperature and humidity);
    c. Solubility (in aqueous and organic solvents); and
    d. Physical properties including melting point (solids), boiling point (liquids), vapor pressure, and flammability.

2. This section should also identify in detail the test agent used to generate the test atmospheres:

   a. Its composition;
   b. Physical Properties;
   c. Source of procurement; and
   d. Batch or lot number of each procurement.

3. Any vehicle or carrier used to enable the test material to be generated should be described in detail.

C. Experimental Design

This section should describe the methods for assigning animals to the different test groups including the controls, the low, middle, and high test groups; the rationale for the levels chosen; the planned interim sacrifices; etc., consistent with the test Guidelines (§82-4).

D. Test Exposures

1. Exposure Chambers

This section should provide sufficient information on the exposure chambers to allow the reviewer to evaluate the suitability of the chambers used. This should include such information as the construction, the dimensions, and sources of the chambers used. If the chambers are not commercially available ones, then information should be provided on the design features and preliminary testing which ensures uniform distribution of air within the chambers. Any alarm systems and automatic recording devices need to be described. The source of make-up air including treatment (filtration, heating and cooling, and humidity control) should be described.

2. Chamber Monitoring

This should include information on the measurements of flow through the chambers, their temperature and relative humidity. Concentration and particle sizing are considered below. The equipment used to measure these parameters needs to be identified as to the vendor, model number, and the method of measurement and recording. Any procedures and equipment used to calibrate the equipment used should be described. The frequency of measurement will depend on the type of study—whether it is a subchronic study or a repeat exposure study.

3. Generating System

The generating systems used for introducing test materials into chambers will vary with the physical state of the test

agent and can involve a wide variety of devices ranging from simple metering devices for gases, evaporating dishes or bubbling bottles for liquid vapors, and nebulizers for liquid aerosols, to particle generators for solid aerosols. The equipment used (vendor, model numbers, dimensions, construction details, etc.), should be described. Problems that arise during development and calibration of the generating system should be discussed in some detail, especially if difficulty is encountered in attaining the necessary concentrations within the chambers.

4. Chamber Concentrations

This should include:

a. The sampling sites within the chamber, the sampling devices used, and the frequency of analyses.
b. The analytical methods used to determine the concentrations of the test agent within the chamber should be discussed in detail (with references).
c. The sampling and analytical equipment used should be described as to vendor, model, source, etc.
d. Any chemicals used in the process should be described as to purity, source, preparations and the like.
e. Information should be provided on the overall precision, accuracy, specificity, and reliability of the chosen methods of analyses for chamber concentrations.

5. Particle Size Determination

Particle size determinations need to be made when test atmospheres are generated as aerosols (sprays or dusts). A description of the methods and equipment used to characterize the aerosols should include such information as the sampling sites, the sampling system, sampling frequency and duration, and the instruments for particle sizing (usually cascade impactors), giving vendors, models, sources, etc. Details on the methods for calibrating and standardizing the sampling flow rates need to be included.

E. Evaluation Procedures

The Final Report should describe the types of observations of the animals. This should include the types of observations and analyses as described in the Guidelines (§82-4(9) through (14)) and should therefore include the frequency of observations, body weights, food consumption, mortality, the hematological examinations, clinical chemistries, urinalysis, gross pathological examination procedures, organ weight measurements and histopathological examination determined at each of the sacrifice times. The statistical analyses used to assess the data for each of the parameters evaluated should be discussed and referenced.

F. Deviations from Protocol

A description of the rationale for deviations from the protocol recommended in §82-4 should be provided along with the rationale for changes made.

IV. RESULTS

This section should provide a systematic summary of the observations made throughout the study summarizing the exposure conditions within each of the chambers and the effects each exposure regimen had on the animals. The data should be presented in graphic and tabular form whenever appropriate, indicating the statistical significance between exposure groups.

V. DISCUSSION

This section should provide an assessment of the results of the study, an interpretation of toxicity observed, the consistency of the dose-response effects and should try to explain unexpected findings. This should include the relationship between the concentration of the test substance and duration of exposure, and the presence or the absence, the incidence and severity, of abnormalities, including behavioral and clinical abnormalities, gross lesions, identified target organs, body weight changes, effects on mortality, and other general and specific toxic effects. This should include the basis for ascribing no-observable-effect and lowest-effect levels when observed. The impact of any deviations from the expected protocol should be discussed including as assessment of the adequacy of the test for the intended purposes.

VI. BIBLIOGRAPHY

This item should contain a list of references cited in the body of the report.

VII. VERIFICATION

This item shall contain information required by §80-4(b)(2). Each test report shall be:

A. Signed by each of the senior scientific personnel, including the laboratory director responsible for performing and supervising the testing, preparing, reviewing, and approving of the test report; and

B. Certified by the applicant or an authorized agent of the applicant as a complete and unaltered copy of the report provided by the testing laboratory whether independent or owned, operated, or controlled by the applicant.

VIII. ARCHIVES

This section of the Final Report shall contain all information required in 40 CFR 160.185.

IX. TABLES/FIGURES

X. APPENDIX(ES)

These should include individual animal data, historical control data, pathology report, analytical methods, and results of analyses on the test substance and test diet, details of statistical analyses, protocol, and other information as appropriate.